IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION OF VISHWANATH R. IYER, Ph.D.
UNDER 37 C.F.R. § 1.132

I, VISHWANATH R. IYER, Ph.D., declare and state as follows:

1.   I am an Assistant Professor in the Section of Molecular Genetics and Microbiology, Institute of Cellular and Molecular Biology, University of Texas at Austin, where my laboratory currently studies global transcriptional control in yeast, gene expression programs during human cell proliferation, and genome-wide transcription factor targets in yeast and human. Immediately prior to this position, I spent four years as a postdoctoral fellow in the laboratory of Patrick O. Brown at Stanford University studying the transcriptional programs of yeast and of human cells. My *curriculum vitae* is attached hereto as Exhibit A.

2.   Beginning in Dr. Brown's laboratory, where I helped to develop the first whole genome arrays for yeast and early versions of highly representative cDNA arrays for human cells, and continuing to the present day, I have used microarray-based gene expression analysis as a principal approach in much of my research.

3.   Representative publications describing this work include:

DeRisi J. *et al.*, "Exploring the metabolic and genetic control of gene expression on a genomic scale," *Science* 278:680-686 (1997);[1]

Marton *et al.*, "Drug target validation and identification of secondary drug target effects using DNA microarrays," Nature Med. 4:1293-1301 (1998);[2]

Iyer *et al.*, "The transcriptional program in the response of human fibroblasts to serum," Science 283:83-87 (1999);[3] and

Ross *et al.*, "Systematic variation in gene expression patterns in human cancer cell lines," *Nature Genetics* 24: 227-235 (2000).[4]

Two of the papers describe our use of microarray-based expression profiling to explore the metabolic reprogramming that occurs during major environmental changes, both in yeast (DeRisi *et al.*, during the shift from fermentation to respiration) and in human cells (Iyer *et al.*, human fibroblasts exposed to serum). One reference describes our use of expression profile analysis in drug target validation and identification of secondary drug effects (Marton *et al.*). And one describes our use of expression profiling as a molecular phenotyping tool to discriminate among human cancer cells (Ross *et al.*).

4. Whether used to elucidate basic physiological responses, to study primary and secondary drug effects, or to discriminate and classify human cancers, expression profiling

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[1] Attached hereto as Exhibit B.

[2] Attached hereto as Exhibit C.

[3] Attached hereto as Exhibit D.

[4] Attached hereto as Exhibit E.

as we have practiced it relies for its power on comparison of **_patterns_** of expression.

5.   For example, we have demonstrated that we can use the presence or absence of a characteristic drug "signature" pattern of altered gene expression in drug-treated cells to explore the mechanism of drug action, and to identify secondary effects that can signal potentially deleterious drug side effects. As another example, we have demonstrated that gene expression patterns can be used to classify human tumor cell lines. While it is of course advantageous to know the biological function of the encoded gene products in order to reach a better understanding of the cellular mechanisms underlying these results, these pattern-based analyses do not require knowledge of the biological function of the encoded proteins.

6.   The resolution of the patterns used in such comparisons is determined by the number of genes detected: the greater the number of genes detected, the higher the resolution of the pattern. It goes without saying that higher resolution patterns are generally more useful in such comparisons than lower resolution patterns. With such higher resolutions comes a correspondingly higher degree of statistical confidence for distinguishing different patterns, as well as identifying similar ones.

7.   Each gene included as a probe on a microarray provides a signal that is specific to the cognate transcript, at least to a first approximation.[5] Each new gene-specific

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[5]   In a more nuanced view, it is certainly possible for a probe to signal the presence of a variety of splice variants of a single gene,

(Continued...)

probe added to a microarray thus increases the number of genes detectable by the device, increasing the resolving power of the device. As I note above, higher resolution patterns are generally more useful in comparisons than lower resolution patterns. Accordingly, each new gene probe added to a microarray increases the usefulness of the device in gene expression profiling analyses. This proposition is so well-established as to be virtually an axiom in the art, and has been as long as I have been working in the field, and certainly since the time I embarked on the production of whole genome arrays in early 1996. Simply put, arrays with fewer gene-specific probes are inferior to arrays with more gene-specific probes.

8.   For example, our ability to subdivide cancers into discriminable classes by expression profiling is limited by the resolution of the patterns produced. With more genes contributing to the expression patterns, we can potentially draw finer distinctions among the patterns, thus subdividing otherwise indistinguishable cancers into a greater number of classes; the greater the number of classes, the greater the likelihood that the cancers classified together will respond similarly to therapeutic intervention, permitting better individualization of therapy and, we hope, better treatment outcomes.

9.   If a gene does not change expression in an experiment, or if a gene is not expressed and produces no

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(...Continued)
without discriminating among them, and for a probe to signal the presence of a variety of allelic variants of a single gene, again without discriminating among them.

signal in an experiment, that is not to say that the probe lacks usefulness on the array; it only means that an insufficient number of conditions have been sampled to identify expression changes. In fact, an experiment showing that a gene is not expressed or that its expression level does not change can be equally informative. To provide maximum versatility as a research tool, the microarray should include -- and as a biologist I would want my microarray to include -- each newly identified gene as a probe.

10. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and may jeopardize the validity of any patent application in which this declaration is filed or any patent that issues thereon.

Vishwanath

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VISHWANATH R. IYER, Ph.D.

October 20, 2003
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Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION OF VISHWANATH R. IYER, Ph.D.
UNDER 37 C.F.R. § 1.132

I, VISHWANATH R. IYER, Ph.D., declare and state as follows:

1.   I am an Assistant Professor in the Section of Molecular Genetics and Microbiology, Institute of Cellular and Molecular Biology, University of Texas at Austin, where my laboratory currently studies global transcriptional control in yeast, gene expression programs during human cell proliferation, and genome-wide transcription factor targets in yeast and human.   Immediately prior to this position, I spent four years as a postdoctoral fellow in the laboratory of Patrick O. Brown at Stanford University studying the transcriptional programs of yeast and of human cells.   My *curriculum vitae* is attached hereto as Exhibit A.

2.   Beginning in Dr. Brown's laboratory, where I helped to develop the first whole genome arrays for yeast and early versions of highly representative cDNA arrays for human cells, and continuing to the present day, I have used microarray-based gene expression analysis as a principal approach in much of my research.

3.   Representative publications describing this work include:

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4. Whether used to elucidate basic physiological responses, to study primary and secondary drug effects, or to discriminate and classify human cancers, expression profiling

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as we have practiced it relies for its power on comparison of **patterns** of expression.

5. For example, we have demonstrated that we can use the presence or absence of a characteristic drug "signature" pattern of altered gene expression in drug-treated cells to explore the mechanism of drug action, and to identify secondary effects that can signal potentially deleterious drug side effects. As another example, we have demonstrated that gene expression patterns can be used to classify human tumor cell lines. While it is of course advantageous to know the biological function of the encoded gene products in order to reach a better understanding of the cellular mechanisms underlying these results, these pattern-based analyses do not require knowledge of the biological function of the encoded proteins.

6. The resolution of the patterns used in such comparisons is determined by the number of genes detected: the greater the number of genes detected, the higher the resolution of the pattern. It goes without saying that higher resolution patterns are generally more useful in such comparisons than lower resolution patterns. With such higher resolutions comes a correspondingly higher degree of statistical confidence for distinguishing different patterns, as well as identifying similar ones.

7. Each gene included as a probe on a microarray provides a signal that is specific to the cognate transcript, at least to a first approximation.[5] Each new gene-specific

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[5] In a more nuanced view, it is certainly possible for a probe to signal the presence of a variety of splice variants of a single gene,

probe added to a microarray thus increases the number of genes detectable by the device, increasing the resolving power of the device. As I note above, higher resolution patterns are generally more useful in comparisons than lower resolution patterns. Accordingly, each new gene probe added to a microarray increases the usefulness of the device in gene expression profiling analyses. This proposition is so well-established as to be virtually an axiom in the art, and has been as long as I have been working in the field, and certainly since the time I embarked on the production of whole genome arrays in early 1996. Simply put, arrays with fewer gene-specific probes are inferior to arrays with more gene-specific probes.

8.   For example, our ability to subdivide cancers into discriminable classes by expression profiling is limited by the resolution of the patterns produced. With more genes contributing to the expression patterns, we can potentially draw finer distinctions among the patterns, thus subdividing otherwise indistinguishable cancers into a greater number of classes; the greater the number of classes, the greater the likelihood that the cancers classified together will respond similarly to therapeutic intervention, permitting better individualization of therapy and, we hope, better treatment outcomes.

9.   If a gene does not change expression in an experiment, or if a gene is not expressed and produces no

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without discriminating among them, and for a probe to signal the presence of a variety of allelic variants of a single gene, again without discriminating among them.

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lacks usefulness on the array; it only means that an
insufficient number of conditions have been sampled to
identify expression changes. In fact, an experiment showing
that a gene is not expressed or that its expression level does
not change can be equally informative. To provide maximum
versatility as a research tool, the microarray should
include -- and as a biologist I would want my microarray to
include -- each newly identified gene as a probe.

10. I declare further that all statements made
herein of my own knowledge are true and that all statements
made on information and belief are believed to be true, and
further that these statements were made with the knowledge
that willful false statements and the like so made are
punishable by fine or imprisonment, or both, under
Section 1001 of Title 18 of the United States Code and may
jeopardize the validity of any patent application in which
this declaration is filed or any patent that issues thereon.

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VISHWANATH R. IYER, Ph.D.

October 20, 2003
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION OF JOHN C. ROCKETT, Ph.D.
UNDER 37 C.F.R. § 1.132

I, JOHN COUGHLIN ROCKETT III, Ph.D., declare and
state as follows:

1. Since 1995 I have been engaged full-time in
molecular toxicology research, with an emphasis on the
application of expression profiling techniques, including but
not limited to nucleic acid microarray expression profiling
techniques, to studies of the mechanisms of toxicant action
and to the design of assays to monitor toxicant exposure.

2. My *curriculum vitae*, including my list of
publications, is attached hereto as Exhibit A.

3. For the past 5 years, my work has focused
primarily on analyzing the effects of potentially hazardous
environmental agents, such as heat, water disinfectant
byproducts, and conazole fungicides on the male reproductive
tract. Although we are interested in the basic mechanisms of
action of such toxicants, we also have two practical goals in
mind: first, to identify individual agents and families of
agents that adversely affect male reproductive development and
function, and second, to develop methods for monitoring human
exposure to such agents, particularly methods capable of
identifying toxicant exposure at an early stage.

4. I have relied on expression profiling as a
principal approach to these goals. Expression profiling, by

reporting the expression levels of thousands of genes simultaneously, gives us an opportunity to identify and group toxicants based on similarities in the _patterns_ of gen expression they induce in cells and tissues; the gene expression profiles induced by treatment with known testicular toxins serve as standards, molecular signatures or molecular fingerprints as it were, against which the patterns of gene expression induced by agents of unknown toxicity may be compared and judged. In addition, gene expression profiling may give us the opportunity to detect toxicity before more gross phenotypic changes become manifest.

5. In keeping with this research emphasis, I have until recently:

served on the Microarray Technical Subcommittee of the United States Environmental Protection Agency (EPA) Genomics Task Force, and

served on the Scientific Committee for the conference series on "Critical Assessment of Techniques for Microarray Data Analysis," held annually at Duke University, Durham, NC;

and I currently

serve on the Technical Committee on the Application of Genomics to Mechanism-Based Risk Assessment of the International Life Sciences Institute's Health and Environmental Sciences Institute,

serve on the Genomics and Proteomics Committee of the National Health and Environmental Effects Research Laboratory of the EPA's Office of Research and Development,

belong to the [North Carolina Research] Triangle Array Users Group,

belong to the Molecular Biology Speciality Section of the Society of Toxicology, and

belong to the Triangle Consortium for Reproductive Biology.

In addition, I am the principal investigator on a cooperative research and development agreement (CRADA) entitled "Development of a Genetic Test for Male Factor Infertility." Prior to this, I was a co-principal investigator on a materials cooperative research and development agreement (MCRADA) to print oligonucleotide-based microarrays; and from 1999 - 2002, I was coinvestigator on a CRADA to develop gene microarrays for toxicology applications.

6. I presume the reader's familiarity with the basic construction and operation of microarrays. For purposes of the discussion to follow, I use the phrase "nucleic acid microarray" and, equivalently, the term "microarray" to refer generically to the various types of nucleic acid microarray that include immobilized nucleic acid probes of sufficient length to permit specific binding, with minimal cross-hybridization, to the probe's cognate transcript, whether the transcript is in the form of RNA or DNA. Although this definition excludes microarrays having shorter probes, such as the 20-mer probes of arrays manufactured by Affymetrix, Inc., many of the comments that follow nonetheless apply to such microarrays as well.

7. Although my own work with microarrays dates back only to 1998, and high density spotted nucleic acid

microarrays themselves date back perhaps only to 1995,[1] microarrays are by no means the only, nor the first, expression profiling tool. As I describe in detail in my *Xenobiotica* review,[2] there are a number of other differential expression analysis technologies that precede the development of microarrays, some by decades, and that have been applied to drug metabolism and toxicology research, including: (1) differential screening; (2) subtractive hybridization, including variants such as chemical cross-linking subtraction, suppression-PCR subtractive hybridization and representational difference analysis; (3) differential display; (4) restriction endonuclease facilitated analyses, including serial analysis of gene expression (SAGE) and gene expression fingerprinting; and (5) EST analysis.

8.   In my own earlier research, I used both reverse-transcriptase polymerase chain reaction (RT-PCR) and suppression-PCR subtractive hybridization (SSH) to study patterns of differential gene expression caused by hepatic challenge with nongenotoxic and genotoxic hepatotoxins.[3]

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[1]   Schena *et al.*, "Quantitative monitoring of gene expression patterns with a complementary DNA microarray," *Science* 270:467-470 (1995), attached hereto as Exhibit B.

[2]   Rockett *et al.*, "Differential gene expression in drug metabolism and toxicology: practicalities, problems and potential," *Xenobiotica* 29:655-691 (1999) (hereinafter, "*Xenobiotica* review"), attached hereto as Exhibit C.

[3]   See, *e.g.*, Rockett *et al.*, "Molecular profiling of non-genotoxic carcinogenesis using differential display reverse transcription polymerase chain reaction (ddRT-PCR)," *European J. Drug Metabolism & Pharmacokinetics* 22(4):329-33 (1997), and Rockett *et al.*, "Use of a suppression-PCR subtractive hybridization method to identify gene species which demonstrate altered expression in male rat and guinea pig livers following 3-day exposure to [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid," *Toxicology* 144(1-3):13-29 (2000), attached hereto respectively as Exhibits D and E.

9.   These older transcript expression profiling techniques provide analogous expression data, but with far lower throughput.

10.   It has been well-established, at least since the introduction of high density spotted microarrays in 1995, that:

(i) each probe on the microarray, with careful design and sufficient length, and with sufficiently stringent hybridization and wash conditions, binds specifically and with minimal cross-hybridization, to the probe's cognate transcript;

(ii) each additional probe makes an additional transcript newly detectable by the microarray, increasing the detection range, and thus versatility, of this analytical device for gene expression profiling;[4]

(iii) it is not necessary that the biological function be known in order for the gene,

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[4]   The compelling logic of this proposition has likely motivated the remarkable rapid progress from the earliest high density spotted arrays in 1995 (Schena et al., "Quantitative monitoring of gene expression patterns with a complementary DNA microarray," Science 270:467-470 (1995), attached hereto as Exhibit B), to the first whole genome arrays in 1997 (Lashkari et al., "Yeast microarrays for genome wide parallel genetic and gene expression analysis," Proc. Natl. Acad. Sci. USA 94(24):13057-62 (1997) and DeRisi et al., "Exploring the metabolic and genetic control of gene expression on a genomic scale," Science 278(5338):680-6 (1997), attached hereto as Exhibits F and G, respectively), to the concurrent announcement by two companies earlier this month of their respective commercial introductions of single chip human whole genome arrays (Pollack, "Human Genome Placed on Chip; Biotech Rivals Put it Up for Sale," The New York Times, Thursday, October 2, 2003 (Business Day), attached hereto as Exhibit H; "Agilent Technologies ships whole human genome on single microarray to gene expression customers for evaluation," Press Release, Agilent Technologies, October 2, 2003, attached hereto as Exhibit I; "Affymetrix Announces Commercial Launch of Single Array for Human Genome Expression Analysis; More Than 1 Million Probes Analyze Expression Levels of Nearly 50,000 RNA Transcripts and Variants on a Single Array the Size of a Thumbnail," Press Release, Affymetrix, October 2, 2003, attached hereto as Exhibit J).

or a fragment of the gene, to prove useful as a probe on a microarray to be used for expression analysis;

(iv) failure of a probe to detect changes in expression of its cognate gene does not diminish the usefulness of the probe on the microarray; and

(iv) failure of a probe to detect a particular transcript in any single experiment does not deprive the probe of usefulness to the community of users who would use this research tool.

These principles also apply to transcript expression profiling techniques that antedate the development of high density spotted microarrays, and accordingly were well-understood prior to 1995.

11. Moreover, expression profiling is not limited to the measurement of mRNA transcript levels. It is widely understood among molecular and cellular biologists that protein expression levels provide complementary profiles for any given cell and cellular state. Although I cannot claim credit for having coined the phrase, I have written that the difference between transcript expression profiling and protein expression profiling is that "transcriptomics indicates what should happen, and proteomics shows what is happening."[5]

12. For decades, such protein expression profiles have been generated using two dimensional polyacrylamide gel

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[5]   Rockett, "Macroresults through Microarrays," *Drug Discovery Today* 7:804 - 805 (2002) (emphasis added), attached hereto as Exhibit K.

electrophoresis (2D-PAGE), and used, among other things, to study drug effects.[6]

13. Although the protein expression profiles produced by 2D-PAGE analysis are analogous to the transcript expression profiles provided by nucleic acid microarrays, an even closer analogy is perhaps offered by antibody microarrays; as I note in my *Drug Discovery Today* commentary, such antibody microarrays date back to the work of Roger Ekins in the mid- to late-1980s.[7]

14. The principles in paragraph 10 also apply to protein expression profiling analyses, particularly to analyses performed using antibody microarrays. Thus, as with nucleic acid microarrays, the greater the number of proteins detectable, the greater the power of the technique; the absence or failure of a protein to change in expression levels does not diminish the usefulness of the method; and prior knowledge of the biological function of the protein is not required. As applied to protein expression profiling, these principles have been well understood since at least as early as the 1980s.

15. Both gene and protein expression profiling are particularly useful to the toxicologist, especially in the pharmaceutical industry. Accordingly, I made the following

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[6] See, e.g., Anderson et al., "A two-dimensional gel database of rat liver proteins useful in gene regulation and drug effects studies," *Electrophoresis* 12:907 - 930 (1991), attached hereto as Exhibit L.

[7] See Ekins et al., *J. Bioluminescence Chemiluminescence* 5:59-78 (1989); Ekins *et al.*, *Clin. Chem.* 37: 1955-1965 (1991); and Ekins, U.S. Patent Nos. 5,432,099, 5,807,755, and 5,837,551, attached hereto respectively as Exhibits M to Q.

statements in my *Xenobiotica* review, written in the summer of 1998:

> [I]n the field of chemical-induced toxicity, it is now becoming increasingly obvious that most adverse reactions to drugs and chemicals are the result of multiple gene regulation, some of which are causal and some of which are casually-related to the toxicological phenomenon *per se*. This observation has led to an upsurge in interest in gene-profiling technologies which differentiate between the control and toxin-treated gene pools in target tissues and is, therefore, of value in rationalizing the molecular mechanisms of xenobiotic-induced toxicity.

> Knowledge of toxin-dependent gene regulation in target tissues is not solely an academic pursuit as much interest has been generated in the pharmaceutical industry to harness this technology in the early identification of toxic drug candidates, thereby shortening the developmental process and contributing substantially to the safety assessment of new drugs.

> For example, if the gene profile in response to say a testicular toxin that has been well-characterized *in vivo* could be determined in the testis, then this profile would be representative of all new drug candidates which act via this specific molecular mechanism of toxicity, thereby providing a useful and coherent approach to the early detection of such toxicants.

> Whereas it would be informative to know the identity and functionality of all genes up/down regulated by such toxicants, this would appear a longer term goal, as the majority of human genes have not yet been sequenced, far less their functionality determined. However, the current use of gene profiling yields a *pattern* of gene changes for a xenobiotic of unknown toxicity which may be matched to that of well-characterized toxins, thus alerting the toxicologist to possible *in vivo* similarities between the unknown and the standard. . . .

* * *

Despite the development of multiple technological advances which have recently brought the field of gene expression profiling to the forefront of molecular analysis, recognition of the importance of differential gene expression and characterization of differentially expressed genes has existed for many years.

16.   As noted in the preceding excerpt from my *Xenobiotica* review, expression profiling in toxicology studies yield *patterns* of changes that are characteristic of an agent of unknown toxicity, which patterns may usefully be matched to those of well-characterized toxins.

17.   In the context of such *patterns* of gene expression, each additional gene-specific probe provides an additional signal that could not otherwise have been detected, giving a more comprehensive, robust, higher resolution -- and thus more useful -- pattern than otherwise would have been possible.[8]
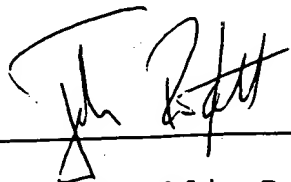
18.   It is my opinion, therefore, based on the state of the art in toxicology at least since the mid-1990s -- and as regards protein profiling, even earlier -- that disclosure of the sequence of a new gene or protein, with or without knowledge of its biological function, would have been

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[8]   In a sense, each gene-specific probe used in such an analysis is analogous to a different one of the many parts of an engine, with each individual part, or subcombinations of such parts, deriving at least part of their usefulness from the utility of the completed combination, the functioning engine.

sufficient information for a toxicologist to use the gene and/or protein in expression profiling studies in toxicology.

19. The statements made in this declaration represent my individual views and are not intended to represent the opinion of my employer, the United States Environmental Protection Agency, or of any other branch of the federal government. Other than my current engagement to provide this declaration, I have neither had, nor currently have, financial ties to, or financial interest in, Incyte Corporation. I am not myself an inventor on any patent application claiming a gene or gene fragment.

20. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and may jeopardize the validity of any patent application in which this declaration is filed or any patent that issues thereon.

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John Coughlin Rockett III, Ph.D.

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